Emirates Journal of Food and Agriculture. 2023. 35(8): 708-714
doi: 10.9755/ejfa.2023.v35.i8.3129
http://www.ejfa.me/

RESEARCH ARTICLE

Evaluation of cytogenotoxic effect of potassium acetate on *Allium cepa* L. root tips

Nasreen Jalal Hussein*

Department of Biology, Faculty of Science, University of Zakho, Zakho, Kurdistan Region-Iraq

ABSTRACT

Potassium acetate is a food additive used for preserving and regulating acidity (buffering agent) of processed food. The continuous use of food additives in our various foodstuffs made it necessary to test their possible toxicity. This research aims to test the cytogenotoxic effects of the food additive potassium acetate (E261) by using *Allium cepa* test system. The parameters tested were: root length, root number, mitotic index, and mitotic abnormalities. The concentrations of potassium acetate were: 0,00% (control), 0,05%, 0,10%, 0.15% 0,20%, and 0,25%. The onions were incubated in different concentrations of potassium acetate for 72 hours. The roots were then taken and spread on a microscopic slide by squash method. The results showed that the tested food additive had a positive effect on root length, root number, and mitotic index at 0.05%. However, it had mitodepressive effect at higher concentrations and also decreased root length and root number as compared to the control. Total mitotic abnormalities increased with increasing the food additive concentration. The different mitotic abnormalities observed were laggard chromosomes, C-mitosis, multipolar anaphase, sticky metaphase, binucleate, sticky anaphase, and micronuclei. The highest effect of the test material was observed at 0.25% potassium acetate. These results suggest that this food additive is mutagenic and can be harmful if used in high concentrations in food.

Keywords: Food additive, Meristematic cells, Mitotic index, Abnormalities

INTRODUCTION

The growing population has increased the demand for different ways to preserve food, and enhance its flavor and color (Olivas and Barbosa-Cánovas, 2005). Food additives are used to preserve, blend, thicken, flavor, and color food (Mpountoukas et al., 2008). Others are used for buffering the acidity of the food. Most of these food additives have a negative effect on our health if consumed in high quantities or prolonged time (Abdulmumeen et al., 2012). Many studies indicated a positive correlation between the mutagenic effects of chemicals and the risk of cancer (Mahapatra and Parija, 2018). Some of the food additives are used to buffer the acidic nature of foods (Lindsay, 2007). Studies indicate that these chemicals are mutagenic, which increases our chances of developing cancer (Moore and Chen, 2006; Prado-Ochoa et al., 2020). Potassium acetate is used in processed food as a preservative and acidity regulator. In the European Union, it is labeled by the E number E261, (UK Food Standards Agency, 2011). Potassium acetate, linear Formula is CH3COOK, (National Center for Biotechnology Information, 2023). Potassium acetate is typically found in such products as sauces and pickles (UK Food Standards Agency, 2011). Plant test systems are commonly used to assess genetic abnormalities induced by various pollutants (Kaya, 2021). Many explorers employ the *A. cepa* test as bioindicators in chemical effect assessments (Bagatini et al., 2009; Leme and Marin-Morales, 2009; Bonciu et al., 2018). Because the *Allium cepa* test may predict potential DNA damage in eukaryotes, it's a useful method for testing mutagenicity (Tedesco and Laughinghouse, 2012). Fiskesjö, (1985) found that the *A. cepa* test correlates well with mammalian test systems. Many researchers used the Allium cepa L as a test system to test mutagenic or the anti-mutagenic effects of different food additives, chemicals, pesticides, and herbicides. For instance, a research by Farheen and colleagues in 2021 investigated the genotoxic effects of four food color additives on the model plant *Allium cepa* root tip cells (Farheen et al., 2021). In 2012, Onyemaobi and colleagues tested the cytogenetic effects of food preservatives, sodium meta-bisulfate, and sodium benzoate on the root tips of *Allium cepa* L. A recent study by Yalçin and Çavuşoğlu in 2022 investigated the toxicity of potassium bromate in *Allium cepa* root tip meristematic

***Corresponding author:**
Nasreen Jalal Hussein, Department of Biology, Faculty of Science, University of Zakho, Zakho, Kurdistan Region-Iraq.
E-mail: nasreen.hussein@uoz.edu.krd

Received: 30 March 2023; **Accepted:** 13 June 2023

cells. In addition, they tested the toxicity-reducing effects of grape seed extract (Yalçin and Çavuşoğlu, 2022). Another recent research used the plant test system *Allium cepa* to test the antioxidant activity of lycopene against dithane toxicity (Macar et al., 2023).

To the best of the author's knowledge, no published data is available that tests the mutagenic effects of the food additive Potassium acetate on *Allium cepa* root tip cells. Therefore, the aim of this study is to evaluate the possible cytotogenotoxic effects of the food additive Potassium acetate on *Allium cepa* root tip meristematic cells. The different parameters tested were: root length, root number, mitotic index, and various mitotic aberrations in *Allium cepa* root tip meristematic cells and their chromosomes.

MATERIALS AND METHODS

Materials

Onion bulbs *(Allium cepa,* 2n= 16 chromosomes) free from fertilizers were purchased from a local store in Zakho/Duhok/Iraq. Potassium acetate powder was obtained from ROHA/India (SRNO: 021, LOT NO: RP19050161), CAS number: 127-08-2. The concentrations of the test material were 0.00% (negative control), 0.05%, 0.10%, 0.15% 0.20% and 0.25%. The different concentrations of Potassium acetate were diluted with distilled water.

Methods

The root number and root length calculation

The onion bulbs were put in distilled water until the root length reached about 1.0 cm then they were transferred and seated on beakers containing different percentages of the test material, after 72 hours they were taken out and about 3 well-germinated bulbs were taken and the root number counted and the root length calculated by a millimetric ruler (Fiskesjö, 1985). The poorly germinated bulbs were discarded.

Preparation of onion root tip mitotic slides

Bulbs of *A. cepa* were placed in small jars with their basal ends dipping in distilled water and germinating at room temperature (25 ± 2 °C). When the newly emerged roots were 1–2 cm in length, they were used in the test. Roots of A. cepa were treated with a series of concentrations of the test substance (potassium acetate). Another group of the bulbs of *Allium cepa* L. were seated on 25cm^3 beakers filled with tap water until the root length reached about 1 cm in length then put in the test substance. The roots were harvested after 72 hours.

The roots were washed with distilled water, cut about 1 cm and kept in Carnoy's fixative. Then the mitotic slides were prepared by Fuelgen Giemsa squash technique and the slides were examined under 1000x light microscopy (Sharma and Sharma, 1999).

Mitotic index calculation

Three slides of each treatment and about 4500 cells from each slide were counted and the mitotic indices were measured. The MI% was calculated according to the formula published by Fiskesjö, (1985).

$$M.I.= DC/TC *100 \quad (1)$$

Where
M.I.= Mitotic Index
DC=Dividing Cells
TC=Total No. of Cells.

Mitotic abnormalities percentage

The abnormalities percentage was calculated according to the following formula

$$A\%= ADC/TC*100 \quad (2)$$

Where
A%=Abnormalities percentage
ADC=Abnormally dividing cells
TC=Total no. of cells.

Statistical analysis

All statistics were done by Statistical Package for Social Scientists software (SPSS version 14) One-Way ANOVA, these were arranged in randomized complete blocks design (RCBD) with 3 replicates, the significant differences were determined between the means of different treatment groups by Duncan's Multiple Range Test (Duncan, 1955). Statistical significance was assumed at $P < 0.05$. Data were reported as mean ± SE. The histogram figures were created by GraphPad Prism software version 8.

RESULTS AND DISCUSSION

Effect on root number and root length of onion bulbs

The root number and root length of different treatments are shown in Fig. 1 and Table 1. According to Table 1, the highest root number and root length were observed at 0.05% potassium acetate and the lowest root number and root lengths were observed at 0.25%. The test material had a positive effect at a very low concentration (0.05%) as compared with the control group. However, at higher concentrations, it gradually reduced root growth and decreased its root number as compared with the control. There were significant differences between treatments in both root number and root length according to DMRT (Fig. 2). The F value was significant at $p<0.01$ for root number and at $p<0.001$ for root length.

Fig 1. The root length of *Allium cepa* after 72 hours treatment with different concentrations of potassium acetate: a- negative control, b- 0.05%, c- 0.10%, d- 0.15%, e- 0.20%, f-0.25%.

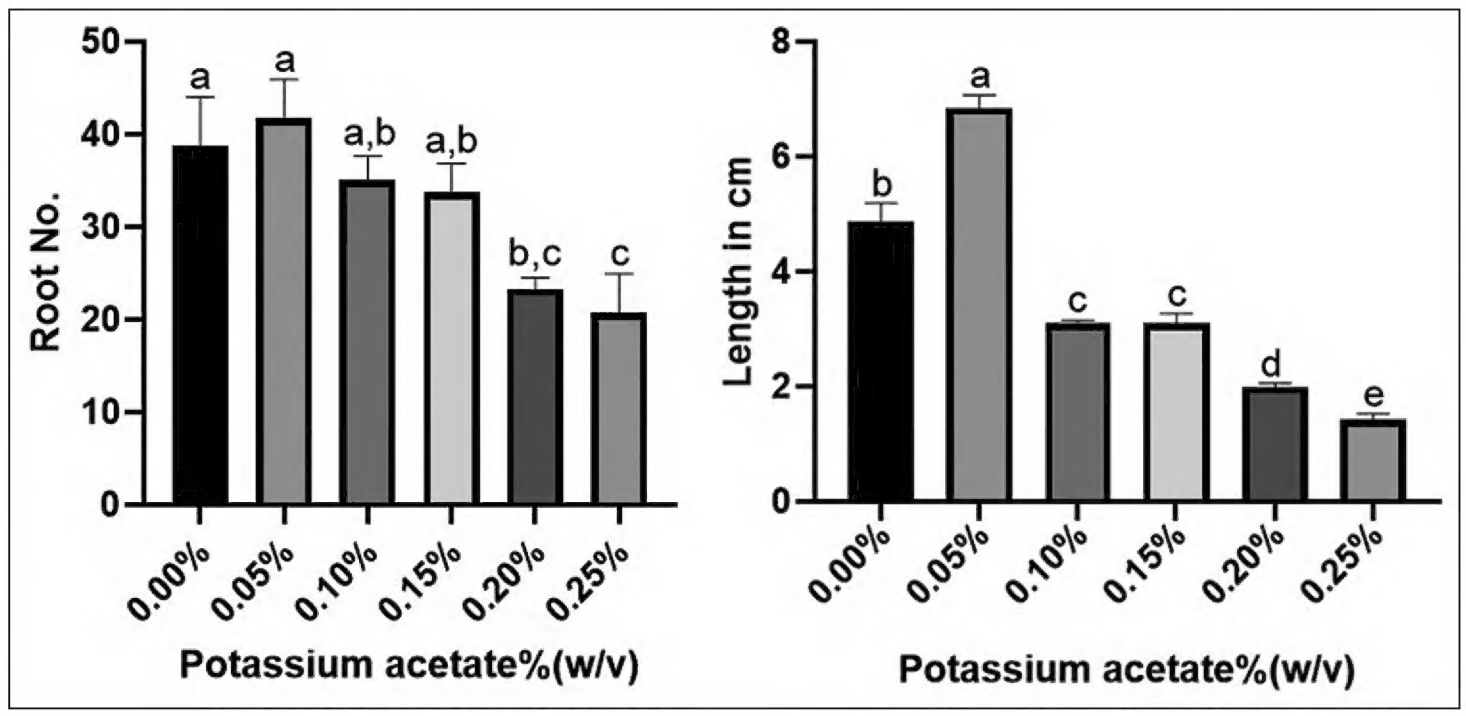

Fig 2. The effect of Potassium acetate on *Allium cepa* root no. and root length.

Table 1: The root number and root length of *Allium cepa* after 72 hours of treatment with different concentrations of Potassium acetate

Treatment%	Root no. (mean±SE)	Root length (mean±SE)
0.00	38.67±5.36^{a}	4.8733±0.32^{b}
0.05	41.67±4.26^{a}	6.8433±0.23^{a}
0.10	35.00±2.65^{a,b}	3.0967±0.05^{c}
0.15	33.67±3.18^{a,b}	3.1000±0.17^{c}
0.20	23.33±1.20^{b,c}	1.9967±0.06^{d}
0.25	20.67±4.26^{c}	1.4200±0.11^{e}
Significance	**	***

= significant at P<0.01, *= significant at P<0.001; superscripts are different letters produced by Duncan's multiple range test.

Effect on mitotic index

About 4500 cells were counted from each treatment, and each treatment was repeated at least three times to count the mitotic index. Table 2 shows the mitotic index of *Allium cepa* root tip after 72 hours treatment. The mitotic index was about 9.7 at control but increased to 13.8 at 0.05% potassium acetate treatment but at higher concentrations decreased until reached 1.8% at 0.25% treatment. The differences were significant at $p<0.001$ at all concentrations. There were also differences within treatments as produced by Duncan's multiple range test (Fig. 3).

The increase in the mitotic index at 0.05% treatment indicates that this substance is not toxic at low concentrations. Dose-dependent inhibition of the mitotic index is in agreement with Dönbak et al., 2002. They tested the genotoxic effects of boric acid on *Allium cepa* test system. Koç and Pandir, (2018) tested the effect of food colors brilliant blue and sunset yellow on *Allium cepa* root tips. Genotoxicity of textile dyeing industry effluents tested on *Allium cepa* by Rahman and his colleagues, (Rahman et al., 2017).

Table 2: Effect of potassium acetate on different mitotic stages and mitotic index of *Allium cepa* root tip cells after 72 hours of treatment

Treatment%	Total cells	Prophase	Metaphase	Anaphase	Telophase	Mitotic index%
0	4509.7±5.84	291.0±7.00^{a}	30.9±5.10^{b,c}	77.3±4.63^{a}	39.7±1.76^{e}	9.7±0.38^{b}
0.05	4518.0±5.57	129.6±0.87^{b}	147.2±0.62^{a}	71.5±0.87^{a}	274.0±2.08^{a}	13.8±0.07^{a}
0.10	4502.3±2.40	89.17±1.30^{c}	37.0±0.58^{b}	36.1±0.47^{b}	67.5±0.76^{b}	5.1±0.03^{c}
0.15	4531.0±1.00	69.67±0.71^{d}	28.0±0.58^{c,d}	30.0±0.58^{c}	53.0±0.58^{c}	4.0±0.05^{d}
0.20	4538.7±5.24	59.63±0.20^{e}	22.3±0.88^{d,e}	22.0±0.58^{d}	45.3±0.88^{d}	3.3±0.05^{e}
0.25	4526.7±2.96	25.53±1.07^{f}	20.3±0.67^{e}	19.0±0.58^{d}	17.3±0.88^{f}	1.8±0.04^{f}
Significance		***	***	***	***	***

: ***= significant at P<0.001; superscripts are different letters produced by Duncan's multiple range test.

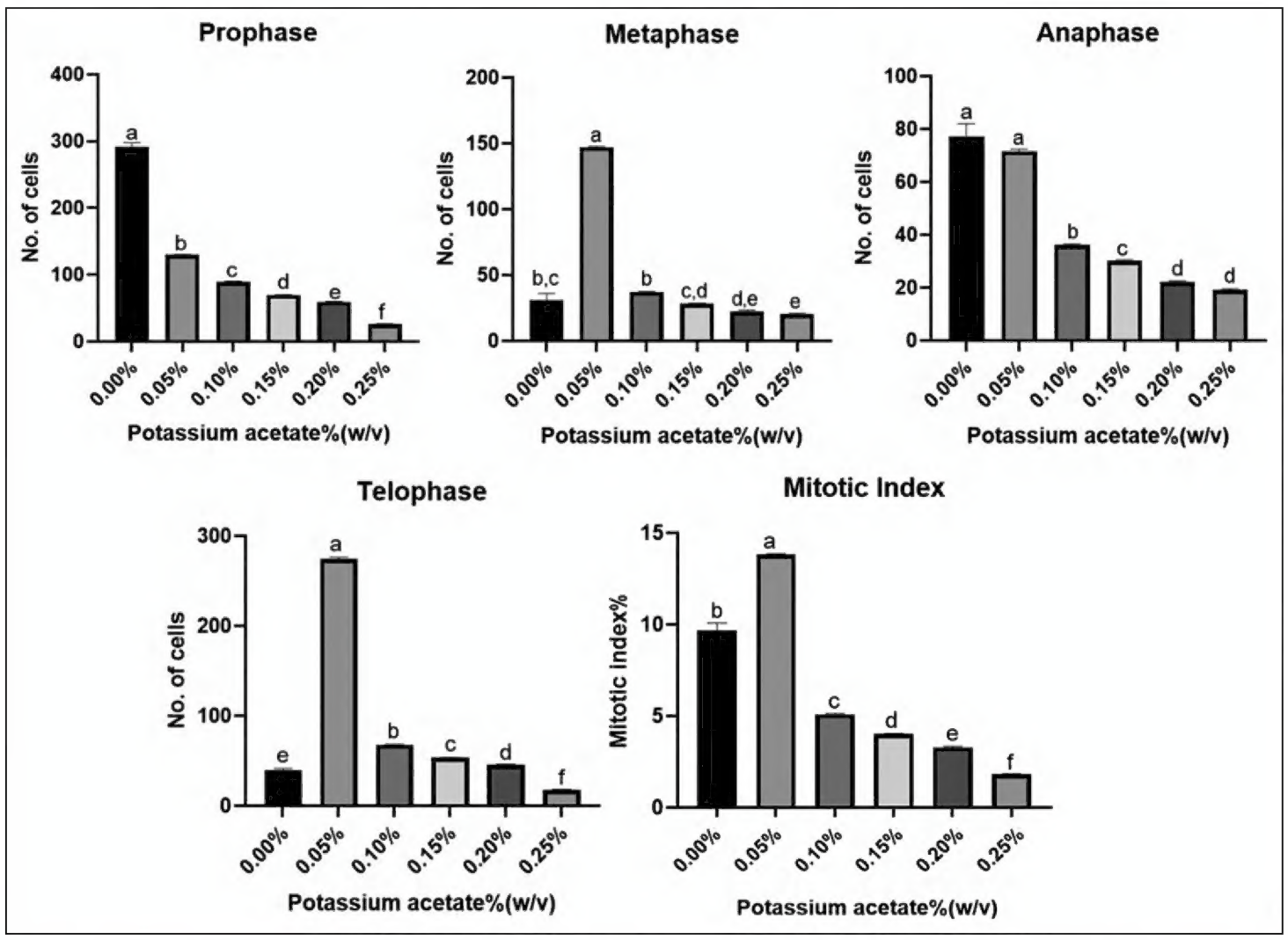

Fig 3. The effect of potassium acetate on different mitotic phases and mitotic index of *Allium cepa* root tip cells.

Effect on mitotic abnormalities

The normal cell before entering mitosis is in interphase, the phase of the cell cycle when the cell is preparing for mitosis but not actually dividing. After the cell begins mitosis, it undergoes through the four major mitotic phases in cells including prophase, metaphase, anaphase and telophase, Fig. 4 shows normal cells at both interphase and different mitotic phases. However, the effect of the test material at different concentrations caused a range of mitotic abnormalities which are shown in Fig. 4. The mitotic aberrations included laggard chromosomes, c-mitosis, multipolar anaphase, sticky metaphase, binucleated cells, sticky anaphase, chromosome loss, and micronuclei.

Table 3 and Fig. 5 show the different mitotic aberrations caused by different concentrations of potassium acetate after 72 hours of exposure. The results show that the mitotic abnormalities increased with dose increase as compared with the control group. There were significant differences between different treatments and all abnormalities were significant at p<0.001. The least frequent abnormality was laggard chromosomes which was 0.01% in the control group and increased to 0.15% at 0.25% treatment, but the most frequent abnormality was the micronuclei which was 0.00% in the control group and increased to 0.53% at 0.25% treatment with potassium acetate. The total abnormalities percentage increased with increasing dose concentration, it increased from 0.04% in the control group and increased to 2.45% at 0.25% after 72 hours of exposure.

The induction of different abnormalities in the *Allium cepa* meristematic cells indicates its potential ability of mutagenic activity. Micronuclei induction and stickiness are the most obvious cytotoxicity indicators. As it is obvious the micronuclei frequency increase was dose-dependent. These results are in agreement with results obtained by (Feretti et al., 2007). The total abnormality percentage increase was

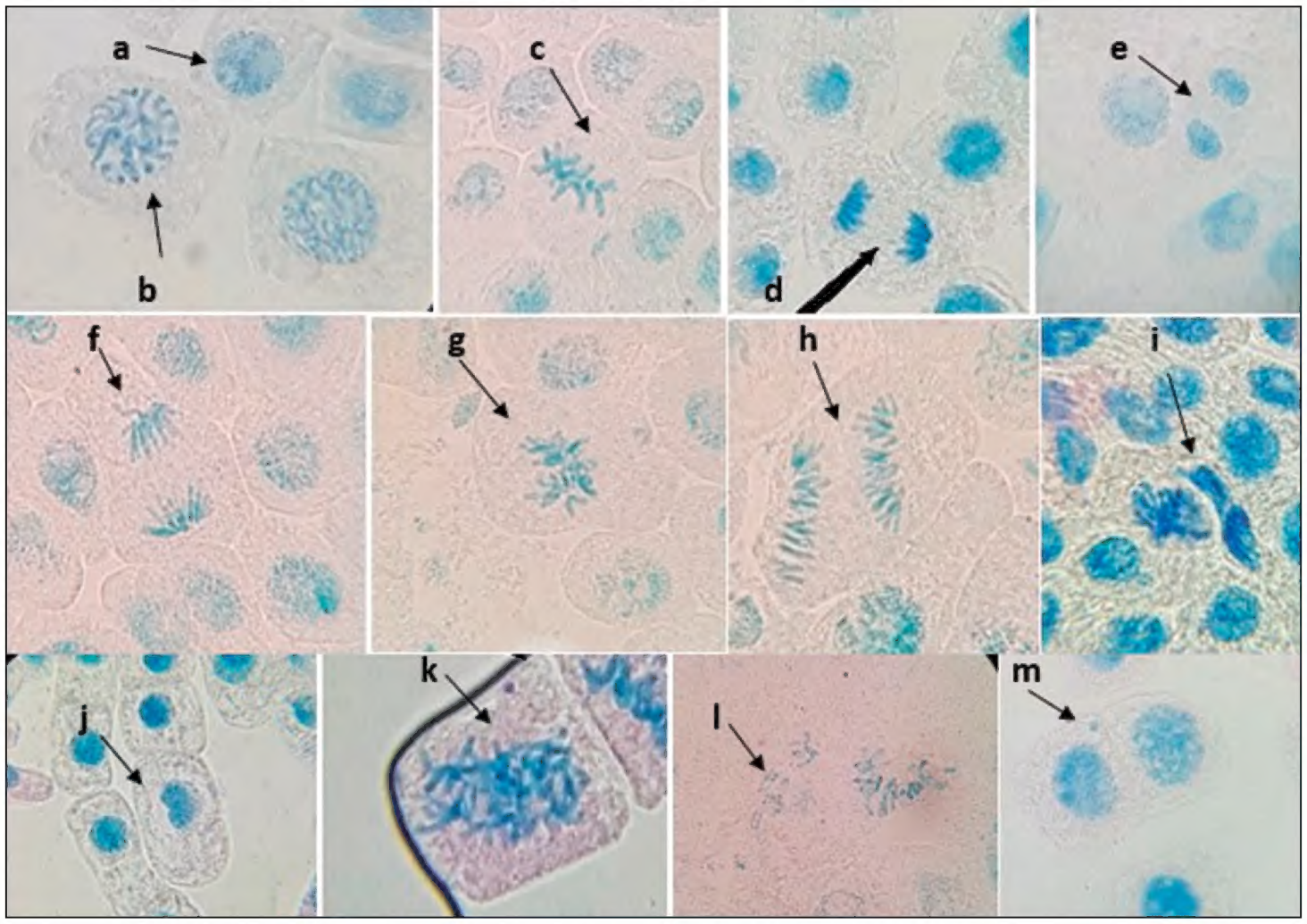

Fig 4. Squash preparations of *Allium cepa* root tip cells: a- interphase, b- normal prophase, c- normal metaphase, d- normal anaphase, e- normal telophase, f- laggard chromosomes, g- C-mitosis, h- multipolar anaphase, i- sticky metaphase, j- binucleated cell, k- sticky anaphase, l- chromosome loss, m- micronucleus.

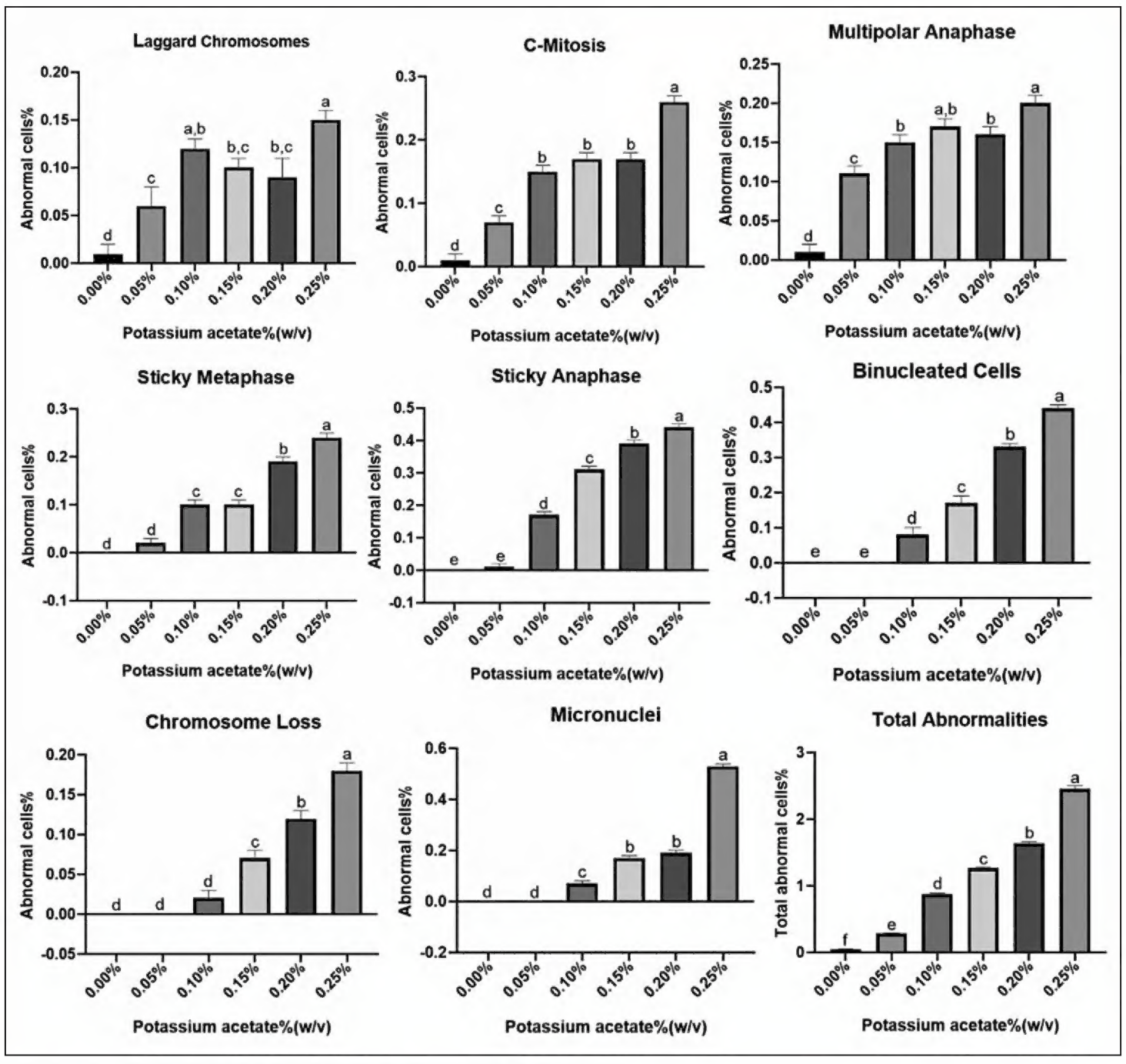

Fig 5. The different mitotic abnormalities caused by Potassium acetate.

Table 3: Mitotic abnormalities% caused by potassium acetate in *Allium cepa* root tip cells after 72 hours

Treatment%	Laggard chromosomes	C-mitosis	Multipolar anaphase	Sticky metaphase	Binucleate	Sticky anaphase	Chromosome loss	Micronuclei	Total abnormalities
0	0.01±0.01^{d}	0.01±0.01^{d}	0.01±0.01^{d}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}	0.04±0.01^{f}
0.05	0.06±0.02^{c}	0.07±0.01^{c}	0.11±0.01^{c}	0.02±0.01^{d}	0.00±0.00^{e}	0.01±0.01^{e}	0.00±0.00^{d}	0.00±0.00^{d}	0.28±0.01^{e}
0.10	0.12±0.01^{a,b}	0.15±0.01^{b}	0.15±0.01^{b}	0.10±0.01^{c}	0.08±0.02^{d}	0.17±0.01^{d}	0.02±0.01^{d}	0.07±0.01^{c}	0.87±0.02^{d}
0.15	0.10±0.01^{b,c}	0.17±0.01^{b}	0.17±0.01^{a,b}	0.10±0.01^{c}	0.17±0.02^{c}	0.31±0.01^{c}	0.07±0.01^{c}	0.17±0.01^{b}	1.26±0.02^{c}
0.20	0.09±0.02^{b,c}	0.17±0.01^{b}	0.16±0.01^{b}	0.19±0.01^{b}	0.33±0.01^{b}	0.39±0.01^{b}	0.12±0.01^{b}	0.19±0.01^{b}	1.63±0.03^{b}
0.25	0.15±0.01^{a}	0.26±0.01^{a}	0.20±0.01^{a}	0.24±0.01^{a}	0.44±0.01^{a}	0.44±0.01^{a}	0.18±0.01^{a}	0.53±0.01^{a}	2.45±0.05^{a}
Significance	***	***	***	***	***	***	***	***	***

***= significant at P<0.001; superscripts are different letters produced by Duncan's multiple range test.

also dose-dependent. This increase is in agreement with previous results of testing various substances on onion root tips (Fatma et al., 2018; Das et al., 2021; Kumar et al., 2022).

CONCLUSION

To accurately assess the possible dangers of cytotoxic agents inherent in the components of these food additives, more research is needed with a variety of doses, exposure durations, and test organisms. These findings will be useful in advising committees responsible for determining the Acceptable Daily Intake (ADI), such as the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), on the establishment of appropriate tolerable limits in the use of this food additive.

Author's contributions

The author NJH designed the study, handled the plants, carried out all experimental work, performed data analysis and interpretation, designed the figures, and also wrote and finalized the manuscript.

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